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(51) International Patent Classification ⁶: C12N 15/82, 15/54, 15/55, 9/10, 9/14, 5/10, C12Q 1/68, G01N 33/50, A01H 5/00	A3	(11) International Publication Number: WO 99/38986 (43) International Publication Date: 5 August 1999 (05.08.99)
(21) International Application Number: PCT/EP99/00556 (22) International Filing Date: 28 January 1999 (28.01.99) (30) Priority Data: 60/109,810 30 January 1998 (30.01.98) US (71) Applicant (for all designated States except AT US): NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH-4058 Basel (CH). (71) Applicant (for AT only): NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H. [AT/AT]; Brunner Strasse 59, A-1235 Vienna (AT). (72) Inventors; and (75) Inventors/Applicants (for US only): GUYER, Charles, David [CA/US]; 4615 J Hope Valley Road, Durham, NC 27707 (US). JOHNSON, Marie, Ann [US/US]; 408 Heather Drive, Raleigh, NC 27606 (US). VOLRATH, Sandra, Lynn [US/US]; 4225 Pine Oak Drive, Durham, NC 27707 (US). BRUNN, Sandra, Alice [CA/US]; 523 Sioux Lane, San Jose, CA 95123 (US). WARD, Eric, Russell [US/US]; 3761 Bentley Drive, Durham, NC 27707 (US).	(74) Agent: BECKER, Konrad; Novartis AG, Corporate Intellectual Property, Patent & Trademark Dept., CH-4002 Basel (CH). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BI, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 23 September 1999 (23.09.99)	
(54) Title: RIBOFLAVIN BIOSYNTHESIS GENES FROM PLANTS AND USES THEREOF		
(57) Abstract The present invention provides plant riboflavin biosynthesis genes, including a gene that encodes the β subunit of the plant riboflavin synthase enzyme complex (lumazine synthase) and a gene that encodes the bifunctional enzyme GTP cyclohydrolase II/DHBP synthase. Also disclosed are the recombinant production of these plant riboflavin biosynthesis enzymes in heterologous hosts, screening chemicals for herbicidal activity using these recombinantly produced enzymes, and the use of thereby identified herbicidal chemicals to suppress the growth of undesired vegetation. Furthermore, the present invention provides methods for the development of herbicide tolerance in plants, plant tissues, plant seeds and plant cells using the riboflavin biosynthesis genes of the invention.		

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/EP 99/00556

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C12N15/54 C12N15/55 C12N9/10 C12N9/14
C12N5/10 C1201/68 G01N33/50 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOBAYASHI, M., ET AL.: "Isolation of cDNAs encoding GTP cyclohydrolase II from <i>Arabidopsis thaliana</i> " GENE, vol. 160, 1995, pages 303-304, XP002110949 the whole document ---	1,9-20, 43,47-49
X	HERZ, S.W., ET AL.: "biosynthesis of riboflavin in plants. The ribA gene of <i>Arabidopsis thaliana</i> specifies a bifunctional GTP cyclohydrolase II/3,4-dihydroxy-2butanone-4-phosphate synthase - unpublished" EMBL SEQUENCE DATA LIBRARY, 1 October 1997 (1997-10-01), XP002110950 heidelberg, germany accession no. AJ000053 ---	1,9-15, 43,47-49

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☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

 In International Application No
 PCT/EP 99/00556

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HERZ, S.W., ET AL.: "unpublished" EMBL SEQUENCE DATA LIBRARY, 29 October 1997 (1997-10-29), XP002110951 HEIDELBERG, GERMANY accession no. AJ002298 -----	1,9-15, 43,45-49
X	DEPREZ, T., ET AL.: "The Arabidopsis thaliana transcribed genome: the GDR cDNA program" EMBL SEQUENCE DATA LIBRARY, 6 June 1994 (1994-06-06), XP002110952 heidelberg, germany accession no. Z34233 -----	5-7
X	DE LOO, F.J., ET AL.: "expressed sequence tags from developing castor seeds" EMBL SEQUENCE DATA LIBRARY, 18 March 1995 (1995-03-18), XP002110953 heidelberg, germany accession no. T15152 -----	1-3,5,6, 43,45
A	STEMMER W P C: "RAPID EVOLUTION OF A PROTEIN IN VITRO BY DNA SHUFFLING" NATURE, vol. 370, 4 August 1994 (1994-08-04), pages 389-391, XP002912216 ISSN: 0028-0836 -----	21-42

International application No.

Box 1 Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international Application that do not comply with the prescribed requirements to such an extent that no meaningful international Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
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- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims 2-8, 21-31, 44-46, 50-54, 59-61, 65 completely;

Claims 1, 16-20, 43, 58 partially.

Cloning and recombinant expression of Lumazine Synthase from *Arabidopsis thaliana* and use of the enzyme to screen for inhibitors of the same, furthermore engineering of herbicide resistance in transgenic plants.

2. Claims 9-15, 32-42, 47-49, 55-57, 62-64, 66 completely;

Claims 1, 16-20, 43, 58 partially

Cloning and recombinant expression of GTP cyclohydrolase II/DHBP Synthase from *Arabidopsis thaliana* and use of the enzyme to screen for inhibitors of the same, furthermore engineering of herbicide resistance in transgenic plants.